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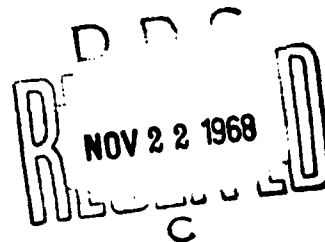
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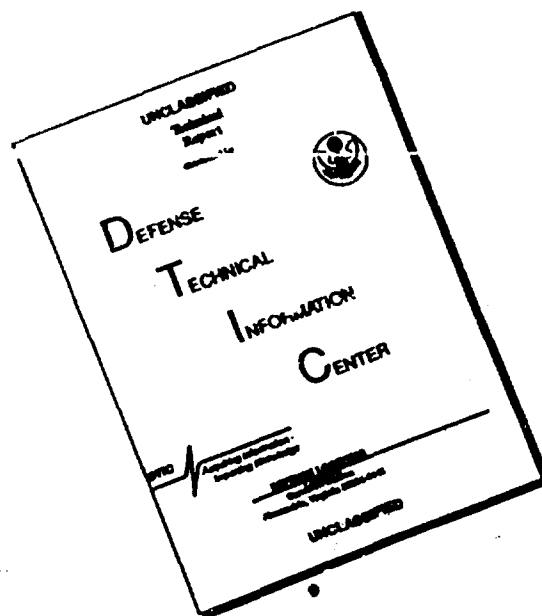
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THE EFFECT OF BACTERIAL TOXINS ON TISSUE CULTURE

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The study of the mechanism of the pathogenic effect of microbial
toxins on an entire organism and its tissues and cells is extremely
important for understanding and specifying the pathogenesis of infectious
diseases. In so doing, the procedure of cultivating tissues and cells
of animals greatly facilitates the study of the problems of infectious
pathology in vitro.

In Soviet and foreign literature there are few well-known works
devoted to the study of the effect of bacterial toxins on tissue cultures
(Saltykov et al., 1961; Penso and Vicari, 1957 and 1958; Felton and
Pomrat, 1962; etc.).

The goal of our work narrowed down to explaining the problem of
whether bacterial toxins act in tissue cultures as selectively as in the
whole organism. A positive solution of this problem would provide an
opportunity for the detailed investigation of the mechanisms specifically
injuring cells and tissues of laboratory models through various toxins.

As models we employed tissue cultures of human, rat, and mouse
embryos and tumoral cells of Erlich's carcinoma. The investigation was
conducted with staphylococcic streptococcic, diphtherial, and tetanic
exotoxins and endotoxins of microbes of the dysenteric-typhous group.

The injurious action of the toxins on the cells of the tissue
culture was determined by the cytotoxic effect resulting from the intro-
duction of various amounts of toxins into the nutrional environment with
the cells. We provisionally individuated, in accordance with the number
of pathologic changes predominant in the cells, four degeneration levels
of the tissue culture: 1) the shape of the cells and nuclei was not changed
and granulation was observed in the cytoplasm; 2) the cells began to lose
their original shapes and cytoplasm was distinctly granular; 3) the uniform

growth of cells was disrupted, they were rounded, the nuclei within them were shriveled, and the cytoplasm was at times vacuolated; and 4) cells degenerated into fragments (detritus) and dropped to the bottom of the test tube.

The following degenerative changes were distinguished in the tumoral cells: 1) sharply pronounced granulation; 2) loss of shape, and granulation located closer to the membrane; and 3) a break in cell membrances and the egress of cytoplasm from cells.

The results of the tests indicated that all the toxins, excepting the tetanic, caused the degeneration of cells of the tissue culture of human embryos in the range of the minimum of the applied amount of toxins (10^{-4}). Stapholoccoccic and streptococcic toxins exerted a harmful influence on the cells within 4 hours, and the remaining toxins, within 24-48 hours. As the amounts of toxins and incubation time increased, changes in the cells became more pronounced. Tetanic toxin, even in large doses and with prolonged action, did not cause any apparent cytotoxic effect in the cells. This corresponded with data contained in literature and confirmed the assumption that this toxin is a specifically neural poison.

The cytotoxic effect of the endotoxins was manifested within a day, and cellular degeneration basically consisted of the appearance of sharply pronounced granulation and vacuolation of cytoplasm, and only on the fourth to the fifth day was the shape of the cells disrupted, their conglutination observed, and nuclear pyknosis begun.

Thus, the dynamics of the pathologic changes in tissue cultures of the fibroblasts of human embryos varied under the influence of different bacterial toxins.

In order to determine whether there were any variations in the sensitivity of different kinds of tissue cultures to the action of microbial toxins, we studied the effect of exotoxins and endotoxins on cultures of the tissues of mouse and rat embryos, as well as on tumoral ascitic cells.

Data obtained from comparative experiments indicated that rat fibroblasts were insensitive to diphtherial toxins, and the mouse fibroblasts were slightly sensitive. This fact concurs with what has long been known regarding the heightened resistance of mice and especially rats to diphtherial toxin.

The endotoxins of the bacteria of the dysenteric-typhous group did not cause any degeneration of the tumoral ascitic cells - - a phenomenon apparently connected with the metabolic characteristics of ascitic cells, as well as with the nature of the chemical structure of endotoxins, which are lipopolysaccharide complexes.

In the whole organism, microbial exotoxins selectively injured specific organs and tissues. We studied the effect of some toxins on the cells of a culture of tissues from various organs of a human embryo - - the intestine and kidneys.

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Table 1 makes evident that diphtherial toxin caused a more pronounced degeneration of cells of renal tissue than the other toxins. The cytotoxic effect of streptococcic toxin and endotoxin on the renal culture was less pronounced than on fibroblasts. Both toxins injured the intestinal tissue culture more severely.

Thus, even in tissue cultures the microbial toxins are observed to be selective in injuring cells of various tissues. The results of these tests confirm the conclusions of a number of researchers (Messore and Caraco, 1957; Gabliks and Zolotorovskiy, 1962).

Table 1

(1) Действие микробных токсинов на культуру ткани почек и кишечника эмбрионов человека

(2) Токсин	(7) Доза токсина	(8) Степень цитотоксического действия в разные сроки на разные ткани							
		(9) 4 часа		(10) 24 часа		(11) 48 часов		(12) 96 часов	
		(13) почек	(14) кишеч.	(13) почек	(14) кишеч.	(13) почек	(14) кишеч.	(13) почек	(14) кишеч.
(3) Стрептококковый	10^{-1}	—	—	+	+	++	++	+++	+++
	10^{-2}	—	—	+	+	++	+	+++	++
	10^{-3}	—	—	—	—	+	+	++	+
	10^{-4}	—	—	—	—	+	—	+	—
(4) Дифтерийный	10^{-1}	—	—	++	+	+++	+	++++	++
	10^{-2}	—	—	++	—	++	+	+++	+
	10^{-3}	—	—	++	—	++	—	+++	—
	10^{-4}	—	—	+	—	++	—	++	—
(5) Дизентерийный Флекснера	10^{-1}	—	—	+	++	+	+++	++	++++
	10^{-2}	—	—	+	++	+	+++	+	++++
	10^{-3}	—	—	—	+	+	++	+	+++
	10^{-4}	—	—	—	+	+	++	+	+++
(6) Брюшнотифозный	10^{-1}	—	—	+	++	+	+++	+	++++
	10^{-2}	—	—	+	+	+	++	+	+++
	10^{-3}	—	—	+	+	+	+	+	+++
	10^{-4}	—	—	—	—	+	+	+	++

(15) Обозначения: ++++, +++, ++, + дегенеративные изменения разной степени, — отсутствие изменений.

Key: (1) Effect of Microbial Toxins on a Culture of Intestinal and Renal Tissue of Human Embryos, (2) Toxin, (3) Streptococcic, (4) Diphtherial, (5) Flexner's dysenteric, (6) Abdominal-typhous, (7) Amount of toxin, (8) Level of cytotoxic effect on various tissues during various periods, (9) 4 hours, (10) 24 hours, (11) 48 hours, (12) 96 hours, (13) kidneys, (14) intestine, (15) the ++++, +++, ++, + signs represent the various degrees of degenerative changes; the - sign indicates the lack of change.

One of the conditions affecting the infectious process is the association of microorganisms in the pathologic foci - - a situation which, perhaps, most frequently intensifies the basic disease. Mixed infections frequently proceed severely, and this distinguishes them from infectious processes caused by a specific single microorganism.

According to the data available in Soviet and foreign literature, the combined effect of various microbial toxins has been inadequately studied. The hemolytic activity of toxins has basically been studied in tests in vitro (Zhak, 1957; Levenshtamm, 1956; Minervin, 1956; Khait, 1960). Some data have been obtained from the study of the joint effect of toxins in vivo (Kirilenko, 1959; Khait, 1962).

We studied the combined effect of a number of exotoxins and endotoxins on tissue cultures of fibroblasts of human embryos and on renal and intestinal tissues. Table 2 indicates that, with the combined action of toxins, their cytotoxic effect on cells is intensified. For example, staphylococcic, streptococcic, and diphtherial toxins taken separately caused no cytotoxic effect on the cells within a four-hour period. Conversely, with the combined action of $\frac{1}{2}$ and $\frac{1}{4}$ of the minimal cytotoxic dose (Dcm), the degeneration of the cells ensued. With the combined action of staphylococcic, streptococcic, and diphtherial toxins ($\frac{1}{2}$ the dose), cytotoxic effect attained the second level within four hours. As a result of the combined action of staphylococcic, streptococcic and diphtherial toxins in the amount of $\frac{1}{2}$ the minimal cytotoxic dose, the second-level degeneration of cells occurred within 4 hours and the third-level degeneration, within 24-48 hours.

Pathologic changes were substantially increased in fibroblasts upon the combined action of exotoxins taken in doses which by themselves did not cause cellular degeneration (1/4, 1/8, and even 1/16 of the minimal cytotoxic dose).

For the purpose of studying the problem of which of the toxins strengthens the cytotoxic effect of another, we arranged the following tests: a dose of one toxin which exerted no cytotoxic influence was introduced into some test tubes with tissue culture ($\frac{1}{2}$ Dcm). This was followed by adding diminishing amounts of another toxin.

The tests indicated that a reciprocal strengthening by the toxins of the cytotoxic effect on the tissue culture did not occur. For example, upon the combined action of the staphylococcic and streptococcic toxins, the intensification of cellular degeneration took place through the streptococcic toxin. The combined action of streptococcic and diphtherial, as well as of staphylococcic and diptherial, toxins caused in the cells a cytotoxic effect which was intensified in both cases under the influence of diphtherial toxin.

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Table 2

(1) Комбинированное действие стафилококкового, стрептококкового
и дифтерийного токсинов на тканевую культуру фибробластов
человеческих эмбрионов

(2) Токсины	(8) Доза токсина (в Dcm)¹			(9) Степень цитотоксического действия в разные сроки (в часах)		
	(10) стафило-кокковый	(11) стрепто-кокковый	(12) дифтерий-ный	4	24	48
(3) Каждый в отдельности	1 ¹/₂ — —	— — 1 ¹/₂ —	— — — 1 ¹/₂	— — — —	+ + + +	++ +++ ++ +++
(4) Стафилококковый и стрептококковый	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	— — — —	+ + ± —	++ + + —	+++ ++ + —
(5) Стафилококковый и дифтерийный	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	— — — —	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	++ + — —	++ + + —	++ ++ + —
(6) Стрептококковый и дифтерийный	— — — —	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	± ± — —	++ ++ + —	+++ ++ + —
(7) Все три токсина	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	¹/₂ ¹/₄ ¹/₈ ¹/₁₀	++ ++ + —	+++ ++ + —	+++ +++ ++ +

(13) Dcm — наименьшее количество токсина, которое вызывало в клетках
цитотоксическое действие через 24 часа.

Key: (1) Combined Effect of Staphyolococcic, Streptococcic, and Diphtherial
Toxins on a Tissue Culture of Fibroblasts of Human Embryos, (2) Toxins,
(3) Each toxin separately, (4) Staphylococcic and streptococcic, (5)
Staphylococcic and diphtherial, (6) Streptococcic and diphtherial (7)
All three toxins, (8) Toxin dose (in Dcm)¹, (9) Level of cytotoxic effect
for various periods (in hours), (10) staphylococcic, (11) streptococcic,
(12) diphtherial, (13)¹ Dcm is the minimal amount of toxin necessary to
cause a cytotoxic effect within 24 hours.

Taking into account the totality of the effect of the endotoxins of the bacteria of the dysenteric-typhous group on the organs and tissues of the whole organism, one would have expected an intensification of the cytotoxic effect on tissue cultures upon the combined action of these toxins. However, the experimental data obtained indicated that the combined action of the endotoxins of the dysenteric-typhous group of microbes did not cause an intensification of the cytotoxic effect in tissue cultures of the fibroblasts of human embryos.

Because a selective sensitivity to some bacterial toxins was observed in specific types of cells in tissue cultures, we thought it would be of interest to study the combined effect of a number of microbe toxins on tissue cultures of the kidneys and intestines of human embryos.

The results of the tests demonstrated that, with the combined action of staphylococcic and diphtherial and streptococcic and diptherial, as well as all the three, toxins, it was possible to observe a cytotoxic effect in the renal tissue culture within four hours. Even such doses of staphylococcic and streptococcic toxins, whose effect on the cells cannot be detected visually (1/4 and 1/8 Dcm), exerted a cytotoxic influence when combined with diphtherial toxin.

Diphtherial toxin intensified the toxic effect: while staphylococcic and streptococcic toxins by themselves did not cause a pronounced degeneration of the renal tissue culture, the addition of diphtherial toxin resulted in significant pathologic changes in the culture.

The above-mentioned data confirmed the selectivity of diphtherial toxin to injure renal tissue of human embryos, since even small doses of it, combined with other exotoxins, stimulated the cytotoxic effect.

In tests run on intestinal tissue culture of human embryos, as well as on fibroblasts, no intensification of cytotoxic effect was observed upon the combined action of endotoxins.

Conclusions

1. Bacterial toxins (excepting tetanic) caused various degrees of degenerative changes in the cells of a tissue culture of human embryos. The dynamics and characteristics of these changes seemed to be heterogeneous, when the effects of exotoxins and endotoxins were compared.

2. Specific and selective effects of bacterial toxins on the cells of various kinds of tissue culture were noted.

3. With the combined action of exotoxins on a culture of human embryo fibroblasts, the cytotoxic effect was intensified, and pathologic changes in the cells occurred before those taking place in the control cells.

4. The combined effect of endotoxins of dysenteric (Flexner) and abdominal-typhous microbes did not intensify degeneration of the tissue culture of human embryo fibroblasts.

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